Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: an open-label dose-ranging study


Methods
In this open-label dose-finding study, we recruited HIV-infected individuals with cryptococcal meningitis who presented to Mulago Hospital in Kampala, Uganda between Aug 14, 2013, and Aug 30, 2014. To assess safety and tolerability, the first 60 participants were given sertraline at escalating doses of 100 mg/day, 200 mg/day, 300 mg/day, or 400 mg/day as induction therapy for 2 weeks, followed by consolidation therapy with 200 mg/day for an additional 8 weeks. From Nov 29, 2013, participants were randomly assigned (1:1) to receive open-label sertraline at predetermined doses of 200 mg/day, 300 mg/day, or 400 mg/day as induction therapy for 2 weeks, followed by consolidation therapy with 200 mg/day for 8 weeks. Dose assignment was made via computer-generated, permuted block randomisation stratified by antiretroviral therapy (ART) status for people with a first episode of meningitis. The primary outcome was 2-week cerebrospinal fluid (CSF) clearance rate of cryptococcus, termed early fungicidal activity, measured in patients with a first episode of culture-positive meningitis and two or more CSF cultures. This study is registered with ClinicalTrials.gov, number NCT01802385.

Findings
Of the 330 individuals assessed, 172 HIV-infected adults with cryptococcal meningitis were enrolled. We gave 100 mg/day sertraline to 17 patients, 200 mg/day to 12 patients, 300 mg/day to 14 patients, and 400 mg/day to 17 patients. 112 participants were randomly assigned to receive sertraline at 200 mg (n=48), 300 mg (n=36), or 400 mg (n=28) daily for the first 2 weeks, and 200 mg/day thereafter. The final population consisted of 17 participants in the 100 mg group, 60 in the 200 mg group, 50 in the 300 mg group, and 45 in the 400 mg group. Participants receiving any sertraline dose averaged a CSF clearance rate of −0.37 colony forming units per mL per day (95% CI −0.41 to −0.33). Incidence of paradoxical immune reconstitution inflammatory syndrome was 5% (two of 43 newly starting ART) and no cases of relapse occurred over the 12-week study period. 38 (22%) of 172 participants had died at 2 weeks, and 69 (40%) had died at 12 weeks. Six grade 4 adverse events occurred in 17 participants receiving 100 mg, 14 events in 60 participants receiving 200 mg, 19 events in 50 participants receiving 300 mg, and eight events in 45 participants receiving 400 mg. Grade 4 or 5 adverse event risk did not differ between current US Food and Drug Administration-approved dosing of 100–200 mg/day and higher doses of 300–400 mg/day (hazard ratio 1.27, 95% CI 0.69–2.32; p=0.45).

Interpretation
Participants receiving sertraline had faster cryptococcal CSF clearance and a lower incidence of immune reconstitution inflammatory syndrome and relapse than that reported in the past. This inexpensive and off-patent oral medication is a promising adjunctive antifungal therapy.

Funding
National Institutes of Health, Grand Challenges Canada.

Introduction
Cryptococcal meningitis has emerged as the most common cause of meningitis in adults in Africa, accounting for 15–20% of AIDS-related deaths.1,2 10-week mortality from cryptococcal meningitis remains unacceptably high (≥35% worldwide),1,2 largely because of the high cost, toxic effects, and limited repertoire of effective antifungal drugs. Furthermore, the rate of fungal clearance of cryptococcus from cerebrospinal fluid (CSF) remains suboptimum, with 60–70% of patients attaining CSF sterilisation after 2 weeks of amphotericin B combination therapy.1,3 For these reasons, a crucial need exists for new effective antifungal drugs that are readily accessible, especially in resource-poor settings.

Evidence suggests that sertraline, the commonly used selective serotonin reuptake inhibitor (SSRI) antidepressant, provides potent in-vitro and in-vivo fungicidal activity against cryptococcus through dose-dependent inhibition of protein synthesis via interaction with eukaryotic translation initiation factor (Tif3).4,5

Summary
Background Cryptococcus is the most common cause of adult meningitis in Africa. We assessed the safety and microbiological efficacy of adjunctive sertraline, previously shown to have in-vitro and in-vivo activity against cryptococcus.

Background
Cryptococcus is the most common cause of adult meningitis in Africa. We assessed the safety and microbiological efficacy of adjunctive sertraline, previously shown to have in-vitro and in-vivo activity against cryptococcus.
Sertraline concentration in blood is subtherapeutic, yet sertraline is concentrated into brain tissue at a median of 16-5-times higher concentrations than in plasma. In vitro, sertraline inhibited Cryptococcus neoformans growth with minimum inhibitory concentrations (MICs) between 2 and 6 μg/mL and unlike fluconazole, sertraline was fungicidal, with killing independent of cell proliferation. The inhibitory effect of sertraline in the brains of infected mice, when treated with sertraline for 7 days before infection, was particularly potent, with efficacy similar to that of fluconazole. The combination of sertraline and fluconazole was either additive or synergistic in vitro, and in mice models led to accelerated fungal clearance at a greater rate than either drug alone. Taken together, these data suggest that sertraline might offer a promising therapeutic option for cryptococcal meningitis.

We postulated that sertraline, when added to standard induction therapy, consisting of amphotericin plus fluconazole, would result in faster rates of fungal clearance from CSF and better clinical outcomes. To test this hypothesis, we assessed the safety, microbiological efficacy, and pharmacokinetics of adjunctive sertraline in HIV-infected Ugandans with cryptococcal meningitis.

Methods

Study design and participants

In this prospective, open-label, dose-finding pilot study, we prospectively enrolled HIV-infected adults (aged ≥18 years) with cryptococcal meningitis, who presented to Mulago Hospital in Kampala, Uganda, between Aug 13, 2013, and Aug 30, 2014. Patients were excluded from participating in the study if they had received more than three doses of amphotericin B, had jaundice or known liver cirrhosis, were pregnant, or were breastfeeding. Due to delay in procuring a suitable matched placebo for the follow-up phase 3 trial, this open-label pilot study was extended to include a so-called mock randomisation of sertraline doses for two purposes: to accrue additional pharmacokinetic and safety data and to provide procedural experience for an anticipated phase 3 randomised trial. This extension began in Nov 29, 2013, and continued until the matched placebo became available on Aug 30, 2014. All study participants provided written informed consent. Uganda and Minnesota institutional review boards approved the protocol.

Procedures

Cryptococcal diagnosis was made via CSF cryptococcal antigen lateral flow assay (Immy Inc, Norman, OK, USA), and confirmed by quantitative CSF fungal culture. Participants received standard antifungal therapy plus adjunctive sertraline at doses of 100–400 mg/day for 12 weeks. Standard therapy included amphotericin B (0.7–1.0 mg/kg per day) for up to 14 days and fluconazole (800 mg/day) for 4 weeks, followed by fluconazole 400 mg/day for 8 weeks of consolidation therapy. Fluconazole dose was increased by 50% in participants who were receiving rifampicin. Amphotericin could be discontinued after 7 days if baseline CSF culture was sterile at 7 days post collection, with continuation of fluconazole and sertraline. For antiretroviral therapy (ART)-naive people or those on a failing regimen, ART was initiated or changed at 4–6 weeks.
At week 9, a 3-week sertraline taper was started, so that participants discontinued sertraline 1 week before study termination at 12 weeks. After 12 weeks, participants were passively followed up and instructed to contact study personnel if they had recurrent CNS symptoms.

To assess safety and tolerability, the first 60 participants were given sertraline at escalating doses of 100 mg/day, 200 mg/day, 300 mg/day, or 400 mg/day as induction therapy for 2 weeks, followed by consolidation therapy with 200 mg/day for 8 weeks. Dose escalation occurred only after five ART-naive individuals with a first episode of cryptococcosis completed 2 weeks of induction therapy. Each dose escalation cohort varied in size according to differences in enrolment pace, 2-week survival, and proportion of people receiving ART. Patients receiving ART at time of enrolment, with a previous history of cryptococcosis, or who died before receiving 14 doses of amphotericin, were included in the overall analysis, but did not count toward the five patients needed to complete each dose cohort prespecified in our protocol.

For the so-called mock randomisation of sertraline doses, beginning on Nov 29, 2013, participants with a first episode of cryptococcal meningitis were assigned to receive open-label sertraline at predetermined doses of 200 mg/day, 300 mg/day, or 400 mg/day as induction therapy for 2 weeks, followed by consolidation therapy with 200 mg/day for 8 weeks. Dose assignment was made via computer-generated, permuted block randomisation stratified by ART status for people with first episode of meningitis. Participants with a second episode of cryptococcal meningitis (eg, relapse or paradoxical immune reconstitution inflammatory syndrome) received 200 mg/day.

Therapeutic lumbar punctures were routinely done using manometers at diagnosis, and on days 3, 7, 10, and 14. Quantitative CSF cultures were done with five serial dilutions (1:10) of 100 μL CSF. Details of the methods used for determining quantitative CSF cultures have been previously described. We defined CSF culture sterility as no growth of cryptococcus, with a limit of detection of 10 colony forming units (CFU) per mL. Cryptococcus isolates were stored in glycerol at –80°C, and shipped on dry ice (–20°C) to the University of Minnesota (Minneapolis, MN, USA). Susceptibility testing was done by broth microdilution in RPMI1640 media per protocol. We defined sertraline MIC as the concentration at which no growth was observed on the basis of photometric absorbance at 600 nm (OD600), as further described.

We quantified plasma sertraline concentrations using reversed-phase liquid chromatography with Agilent 1200 series HPLC pump (Agilent, Santa Clara, CA, USA), auto sampler, and column oven with triple quadrupole mass spectrometer (appendix). We attempted to measure sertraline concentrations directly in CSF, but this measurement was abandoned after discovering very low concentrations in CSF compared with those in plasma. Because sertraline is a highly lipophilic molecule, we postulated that sertraline diffused from CSF into brain tissue, rendering it undetectable in CSF. Indeed, sertraline concentrations in human CSF have never been published. We thus estimated sertraline brain concentrations on the basis of published post-mortem tissue levels, which were shown to have a median 16.5-fold (IQR 13.0–21.3) higher concentration in brain tissue than in plasma.

Outcomes
The primary outcome was the 2-week CSF clearance rate of cryptococcos, termed early fungicidal activity. Secondary clinical endpoints included incidence of CSF culture sterility at 2 weeks, paradoxical immune reconstitution inflammatory syndrome per consensus criteria, culture-positive relapse, and safety up to 12 weeks. Participants with a previous history of cryptococcosis were only included in the analyses for survival, safety, and relapse. We calculated the incidence of paradoxical immune reconstitution inflammatory syndrome in people with a first episode of cryptococcal meningitis, who were ART-naive at baseline and survived to initiate ART, and in those who switched to second-line ART after hospital admission. We used the National Institute of Allergy and Infectious Diseases Division of AIDS toxicity scale, version 2009, to assess adverse events in all participants. Because of an expected 80% incidence of grade 3–5 adverse events with amphotericin B deoxycholate, we only captured grade 4–5 adverse events, which had an expected incidence of 35–40%, dominated by amphotericin-related toxic effects. Additional protocol-specified secondary outcomes of neurocognitive performance, depression, and cost-effectiveness are the focus of future publications. Secondary laboratory endpoints included plasma sertraline concentrations and in-vitro sertraline susceptibility of cryptococcus isolates. Previous genotyping of cryptococcus clinical isolates in Uganda during 2006–12 has determined that more than 99% of clinical isolates are Cryptococcus neoforms var grubii strains.

Statistical analysis
We assessed baseline characteristics across sertraline doses using χ² or Kruskal-Wallis tests as appropriate. We calculated overall and dose-specific CSF clearance rate over 2 weeks (early fungicidal activity) in participants with a culture-positive first episode of cryptococcosis, and at least two quantitative CSF cultures via longitudinal mixed-effect models, which included participant-specific random intercept and random slope. Models used restricted maximum likelihood estimation and an unstructured covariance matrix. We used the Satterthwaite method to determine denominator degrees of freedom for p value estimation. Because multiple cohorts have reported mean early fungicidal activity by patient-specific linear regression, we also estimated early fungicidal activity by linear regression.
We compared secondary outcomes across sertraline doses with χ² or Kruskal-Wallis tests. To assess efficacy of consolidation therapy with sertraline added, we used Cox proportional hazard regression to compare 12-week all-cause mortality between participants who achieved CSF sterility by 2 weeks versus those who did not, in participants who survived 14 days after enrolment. Age-adjusted and sex-adjusted competing-risks regression assessed grade 4 or 5 adverse event incidence between current US Food and Drug Administration (FDA) dosing guidelines of 100–200 mg/day and higher doses of 300–400 mg/day, using the Fine and Grey method where non-adverse event all-cause mortality was a competing risk. We also calculated risk differences between FDA-approved dose groups for grade 4 adverse events and grade 5 cryptococcal-related and non-cryptococcal adverse events separately. For other safety outcomes, we calculated risk differences for cryptococcal and non-cryptococcal related deaths, one or more occurrences of nausea, vomiting, or diarrhoea; premature sertraline dose reduction; premature sertraline discontinuation; and loss to follow-up between dosing of sertraline 100–200 mg/day and higher doses at 300–400 mg/day. We did the analyses using SAS version 9.3 (SAS Institute, Cary, NC) and assessed against type I error lower than 0·05.

We did the pharmacokinetic analyses using non-linear mixed-effects models to simultaneously estimate parameters of a one-compartment pharmacokinetic model by including all available sertraline concentrations from all doses. We used a likelihood ratio test to determine the significance of including ART in the model (χ² test, α=0·05, df=1). A Monte Carlo simulation for 50 000 simulated individuals determined the proportion with projected therapeutic steady state sertraline brain concentrations, based on the distributions of steady state plasma concentrations, published fold-change concentration into the brain,9 and cryptococcus population MICs (appendix). We did pharmacokinetic analyses to determine ART effect in NONMEM version 7.2 (ICON Development Solutions, Dublin, Ireland). This study is registered with ClinicalTrials.gov, number NCT01802385.

Role of the funding source
The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
Of the 330 individuals presenting with suspected meningitis, 172 HIV-infected adults with a positive CSF cryptococcal antigen consented and were enrolled, from Aug 14, 2013, until Aug 30, 2014. Of these, 22 had a previous history of cryptococcal meningitis (appendix). To assess safety and tolerability, the first 60 participants were given sertraline at escalating doses of 100 mg/day (n=17), 200 mg/day (n=12), 300 mg/day (n=14), or 400 mg/day (n=17) as induction therapy for 2 weeks, followed by consolidation therapy with 200 mg/day for 8 weeks (figure 1).

The following 112 participants were randomly assigned to receive sertraline at 200 mg (n=48), 300 mg (n=36), or 400 mg (n=28) daily for the first 2 weeks, and 200 mg/day thereafter. The final population consisted of 100 mg (n=17), 200 mg (n=60), 300 mg (n=50), or 400 mg (n=45) daily for the first 2 weeks, and 200 mg/day thereafter (n=134 survivors).

The demographic and baseline clinical characteristics of the study participants are presented in table 1. By contrast with previous large cohorts of HIV-associated

Figure 1: Trial profile
60 participants were assessed for safety and tolerability of adjunctive sertraline and an additional 112 participants either underwent a so-called mock randomisation of open-label sertraline (n=96; participants with first episode of cryptococcal meningitis) or received 200 mg daily of adjunctive sertraline for a second episode of cryptococcal meningitis (n=16). CSF=cerebrospinal fluid.
cryptococcal meningitis, about half of participants were receiving ART before hospital admission (50% (29%) of 172 patients were receiving efavirenz-containing ART), about a third presented with altered mental status (Glasgow Coma Scale <15), and fewer patients were women than men (table 1). High baseline fungal burdens and raised CSF opening pressure (median 300 mm H2O, IQR 190–480) were common. Of those with a first episode of cryptococcal meningitis, 12 (8%) of 150 had sterile CSF cultures (95% CI 5–16). Apart from previous cryptococcal meningitis, characteristics did not differ significantly between dosing groups (table 1).

Of the 172 individuals who received sertraline, 44 participants (26%) were excluded from early fungicidal activity analysis because of previous cryptococcal meningitis (n=22), sterile diagnostic CSF cultures (n=12), or fewer than two CSF cultures done (n=10). With sertraline, the overall mixed-effect early fungicidal activity was –0.37 log10 CFU/mL per day (95% CI –0.41 to –0.33). There were no significant differences in early fungicidal activity between sertraline doses (table 2).

Overall 2-week mortality was 22% (38 of 172), and 12-week mortality was 40% (69 of 172), and did not differ by sertraline dose group (table 2). Hospital admission duration, 2-week CSF sterility, or symptomatic recurrence (eg, paradoxical immune reconstitution inflammatory syndrome, culture-positive relapse) did not differ across dosing groups. The incidence of paradoxical immune reconstitution inflammatory syndrome was 5% (two of 43) in participants with a first episode of cryptococcosis who were ART-naive and survived to initiate ART or those switched to second-line therapy because of virological failure. No cryptococcal relapse occurred during 12 weeks of follow-up. Two participants developed CSF culture-positive relapse after 12 weeks, with one due to fluconazole non-compliance.

Adverse events and deaths in this critically ill population were common (table 3). The numbers of individual grade 4 adverse events occurring in each dose group were: six events in 17 participants receiving 100 mg sertraline, 14 events in 60 participants receiving 200 mg sertraline, 19 events in 50 participants receiving 300 mg sertraline, and eight events in 45 participants receiving 400 mg sertraline. More broadly, grade 4 or 5 adverse event risk did not differ between current FDA-approved dosing of 100–200 mg/day and higher doses of sertraline.

Table 1: Baseline characteristics by daily sertraline dose

<table>
<thead>
<tr>
<th>Sertraline dose cohort</th>
<th>Sertraline, all (n=172)</th>
<th>Sertraline, p value (n=172)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg (n=17)</td>
<td>200 mg (n=60)</td>
<td>300 mg (n=50)</td>
</tr>
<tr>
<td>Age, years</td>
<td>36 (32–41)</td>
<td>37 (32–43)</td>
</tr>
<tr>
<td>Male sex</td>
<td>11 (65%)</td>
<td>41 (68%)</td>
</tr>
<tr>
<td>Previous cryptococcal meningitis</td>
<td>1 (6%)</td>
<td>16 (27%)</td>
</tr>
<tr>
<td>Receiving antiretroviral therapy</td>
<td>9 (53%)</td>
<td>33 (55%)</td>
</tr>
<tr>
<td>Receiving treatment for tuberculosis</td>
<td>0</td>
<td>2 (3%)</td>
</tr>
</tbody>
</table>

Baseline clinical parameters

| Glasgow Coma Scale score <15 | 6 (35%)       | 25 (42%)                   | 16 (32%)                    | 13 (29%)                    | 60 (35%)                    | 0.58 |
| Weight, kg                  | 52 (40–57)    | 52 (44–61)                 | 50 (47–55)                  | 52 (45–56)                  | 52 (47–57)                  | 0.91 |
| CD4 count, cells/μL         | 19 (7–114)    | 25 (9–54)                  | 16 (6–50)                   | 20 (9–59)                   | 19 (7–57)                   | 0.57 |
| CSF opening pressure, >250 mm H2O | 8/15 (53%) | 26/52 (69%)                | 24/45 (53%)                 | 25/41 (65%)                 | 93/153 (61%)                | 0.40 |
| CSF quantitative culture, log10 CFU/mL* | 4.8 (4.1–5.4) | 4.4 (3.1–5.4) | 4.9 (4.0–5.5) | 4.3 (3.3–5.5) | 4.6 (3.8–5.4) | 0.46 |
| CSF white blood cells ≥5 cells/μL | 9 (53%)       | 15/55 (27%)                | 13/47 (40%)                 | 12/43 (28%)                 | 55/162 (34%)                | 0.14 |

Data are median (IQR), n (%), or n/N (%) when the denominator differs from that stated at the top of each column. p values are for the comparison across all four sertraline dosing groups. IRIS=immune reconstitution inflammatory syndrome. CSF=cerebrospinal fluid. CFU=colony forming units. *Excludes those with sterile culture at diagnosis (n=19); the cultures of four people were unable to be quantified.

Figure 2: Rate of CSF clearance of cryptococcus, by sertraline dose

No significant difference was observed in the early fungicidal activity between doses of sertraline. Due to the small sample sizes of the dose groups and inherent differences in statistical methods of estimating early fungicidal activity (ie, linear regression vs mixed-model using longitudinal repeated measures), the 95% CIs of each sertraline dose group overlap, and the 95% CI should be appreciated instead of any exact point estimate. The appendix provides early fungicidal activity plots by sertraline dose. CFU=colony forming units. CSF=cerebrospinal fluid. GLM=generalised linear model.
300–400 mg/day (hazard ratio [HR] for grade 4 or 5 adverse events 1.27, 95% CI 0.69–2.32; p=0.45). Most grade 4 or 5 adverse events were related to amphotericin toxic effects or AIDS (53 of 59) including anaemia (n=28), electrolyte abnormalities (n=15), cytopenia (n=4), and acute kidney injury (n=2). Cryptococcal-related deaths occurred in 24–25% of participants up to 12 weeks, and non-cryptococcal deaths occurred in 14–17% of enrolled participants (table 2). The appendix provides a summary of adverse events by dose group and line listing of the incident grade 4–5 adverse events.

A mild-to-moderate serotonin syndrome occurred in one participant who unintentionally self-administered 800 mg/day of sertraline for 3 days over a weekend, which was a protocol deviation. Sertraline was held for 3 days, and then restarted at 200 mg/day with an uneventful course thereafter. Overall tolerability was excellent with six (4%) of 150 participants with no previous history of cryptococcal meningitis having early discontinuation.

Of 101 participants with no previous history of cryptococcosis or sterile diagnostic quantitative cultures who survived 2 weeks after enrolment, 68 (67%) achieved CSF sterility by the end of induction therapy, 23 deaths occurred between 2 and 12 weeks, 16 deaths (24%) in 68 participants who were sterile, and seven deaths (21%) in the 33 participants who were not sterile. Unlike previous studies, CSF culture positivity at the end of induction therapy was not significantly associated with mortality (HR 0.88, 95% CI 0.36–2.15; p=0.78; figure 3).

Sertraline concentrations in plasma were quantified in 143 participants. Sertraline reached steady state in plasma by at least day 7, with median levels of 201 ng/mL (IQR 90–300) when taking 200 mg/day and 399 ng/mL (278–560) when taking 400 mg/day during days 7–14 of the induction period.

### Table 2: Outcomes by daily sertraline dose

<table>
<thead>
<tr>
<th>Sertraline dose cohort</th>
<th>100 mg (n=17)</th>
<th>200 mg (n=60)</th>
<th>300 mg (n=50)</th>
<th>400 mg (n=45)</th>
<th>Sertraline, all (n=172)</th>
<th>Sertraline, p value (n=172)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-day CSF sterility*</td>
<td>6/14 (43%)</td>
<td>25/41 (61%)</td>
<td>22/43 (51%)</td>
<td>20/40 (50%)</td>
<td>72/135 (53%)</td>
<td>0.61</td>
</tr>
<tr>
<td>Paradoxical IRIS†</td>
<td>0/3 (0%)</td>
<td>1/14 (7%)</td>
<td>0/15 (0%)</td>
<td>1/11 (9%)</td>
<td>2/43 (5%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Culture-positive relapse</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-week mortality</td>
<td>5/17 (29%)</td>
<td>8/60 (13%)</td>
<td>12/50 (24%)</td>
<td>12/45 (29%)</td>
<td>38/172 (22%)</td>
<td>0.21</td>
</tr>
<tr>
<td>12-week mortality</td>
<td>10/17 (59%)</td>
<td>20/60 (33%)</td>
<td>21/50 (42%)</td>
<td>18/45 (40%)</td>
<td>69/172 (40%)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Sertraline dose cohort Sertraline, all Sertraline, p value

CSF=cerebrospinal fluid. IRIS=immune reconstitution inflammatory syndrome. *Excludes those who started with sterile CSF culture (n=12) or previous history of cryptococcal meningitis (n=22); includes all quantitative culture data collected within 14 days of enrolment in 138 participants given sertraline. **IRIS incidence in individuals with first episode of cryptococcal meningitis, who were antiretroviral therapy (ART)-naive at baseline and who survived to initiate ART, or those who switched to second-line ART after hospital admission; includes possible IRIS cases; no other paradoxical IRIS cases occurred in those excluded (eg, second episodes of cryptococcosis, those already receiving effective ART). †Two culture-positive cases of relapse occurred beyond the 12-week study follow-up period, one in the 100 mg dose group and another with fluconazole non-compliance.

### Table 3: Adverse events and clinical outcomes with adjunctive sertraline for 12 weeks

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Sertraline 100 and 200 mg/day (n=77)</th>
<th>Sertraline 300 and 400 mg/day (n=95)</th>
<th>Absolute risk difference (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of grade 4 adverse events</td>
<td>20</td>
<td>27</td>
<td>-0.04 (-0.09 to 0.16)</td>
<td>0.56</td>
</tr>
<tr>
<td>Total number of grade 5 adverse events</td>
<td>3</td>
<td>9</td>
<td>0.03 (0.03 to 0.08)</td>
<td>0.38</td>
</tr>
<tr>
<td>Grade 5 adverse event, cryptococcal related</td>
<td>1 (1%)</td>
<td>3 (3%)</td>
<td>-0.02 (-0.02 to 0.06)</td>
<td>0.42</td>
</tr>
<tr>
<td>Grade 5 adverse event, non-cryptococcal</td>
<td>2 (3%)</td>
<td>5 (5%)</td>
<td>-0.05 (0.05 to 0.10)</td>
<td>0.26</td>
</tr>
<tr>
<td>Overall 12-week outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptococcal-related mortality</td>
<td>19 (25%)</td>
<td>23 (24%)</td>
<td>-0.0 (0.13 to 0.12)</td>
<td>0.95</td>
</tr>
<tr>
<td>Non-cryptococcal related mortality</td>
<td>11 (14%)</td>
<td>16 (17%)</td>
<td>0.03 (0.08 to 0.13)</td>
<td>0.65</td>
</tr>
<tr>
<td>Nausea, vomiting, or diarrhoea, one event or more</td>
<td>55 (72%)</td>
<td>61 (64%)</td>
<td>-0.12 (0.26 to 0.01)</td>
<td>0.08</td>
</tr>
<tr>
<td>Serotonin syndrome</td>
<td>0 (0%)</td>
<td>1 (1%)*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sertraline dose reduction, all cause</td>
<td>0 (0%)</td>
<td>1 (1%)*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Early sertraline discontinuation†</td>
<td>1/60 (2%)</td>
<td>5/90 (6%)</td>
<td>-0.04 (-0.02 to 0.10)</td>
<td>0.40</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>3 (4%)</td>
<td>2 (2%)</td>
<td>-0.02 (-0.07 to 0.03)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Details by individual dose group are provided in the appendix. Absolute risk difference represents the difference in proportions, with the p value calculated by the Fisher’s exact χ² test. *Protocol deviation by a participant taking 800 mg/day for 3 days. †Excludes participants with a previous history of cryptococcal meningitis (n=17 for the 100–200 mg/day group and n=5 for the 300–400 mg/day group).
concentrations in brain tissue rises from 81% to 97%. 

With 400 mg/day of sertraline, the median concentrations in patients not receiving ART were 397 ng/mL (IQR 211–495) versus 431 ng/mL (246–566) in those receiving ART. Participation in the first episode of cryptococcal meningitis, might improve CSF fungal clearance. In this pilot dose-finding study, participants receiving any sertraline showed an average CSF clearance rate of –0·37 log10 CFU per mL. By comparison, the rate of CSF clearance was –0·30 log10 CFU per mL CSF per day (–0·32 to –0·28) in 208 participants screened for the Cryptococcal Optimal ART Timing (COAT) trial in Uganda and South Africa during 2010–12. This historical cohort received the same background regimen of amphotericin 0·7–1·0 mg/kg per day and fluconazole 800 mg/day without sertraline. Similarly, the CSF clearance rate in 99 participants in Vietnam receiving amphotericin 1 mg/kg per day and fluconazole 800 mg/day was –0·32 (–0·34 to –0·29) log10 CFU per mL CSF per day.

The rate of cryptococcus clearance from the CSF is a powerful method to explore new drug combinations in small phase 2 studies. The confidence intervals in the rate of fungal clearance between those receiving any dose of sertraline and historical controls receiving the same standard antifungal therapy at the same site did not overlap. The comparison between sertraline dose groups did not reveal any overtly, alarming problems with safety or tolerability of the higher sertraline doses; however, the small sample sizes of the individual dose groups preclude

Discussion

Sertraline, when added to standard amphotericin-combination therapy for cryptococcal meningitis, might improve CSF fungal clearance. In this pilot dose-finding study, participants receiving any sertraline showed an average CSF clearance rate of –0·37 log10 CFU per mL CSF per day (95% CI –0·41 to –0·33). By comparison, the rate of CSF clearance was –0·30 log10 CFU per mL CSF per day (–0·32 to –0·28) in 208 participants screened for the Cryptococcal Optimal ART Timing (COAT) trial in Uganda and South Africa during 2010–12. This historical cohort received the same background regimen of amphotericin 0·7–1·0 mg/kg per day and fluconazole 800 mg/day without sertraline. Similarly, the CSF clearance rate in 99 participants in Vietnam receiving amphotericin 1 mg/kg per day and fluconazole 800 mg/day was –0·32 (–0·34 to –0·29) log10 CFU per mL CSF per day.

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sufficient statistical power to detect possibly important clinical differences.

Although the fungal clearance rate provides a relatively objective outcome measure, these results should be interpreted cautiously. Notably, patients receiving ART were excluded in the historical cohort, although neither receipt of ART in this study nor early ART in the COAT trial were statistically associated with early fungicidal activity. The early fungicidal activity observed with amphotericin and fluconazole 800 mg/day in the COAT trial (n=208) was similar to that observed by Day and colleagues (n=99) using an identical statistical methodology. Definitive declarations cannot be given due to the overall small sample size and lack of direct statistical comparison, yet we believe that these observations provide ample justification for a further phase 3 randomised clinical trial to test the use of adjunctive sertraline.

The current standard therapy for cryptococcal meningitis is based on antifungal regimens that are a half-century old, are associated with a range of toxic effects, and are largely inaccessible in areas of the world where they are needed most. For this reason, the discovery of a widely available, non-toxic, and affordable drug effective against cryptococcus would represent a substantial advance in preventing deaths. Sertraline represents a promising adjunct and deserves further investigation in randomised clinical trials.

The safety and tolerability of sertraline was good. The incidence of grade 4–5 adverse events and gastrointestinal side-effects, although seemingly high, were similar to or less than previously reported. During the COAT trial over 12 weeks, there was a 50% cumulative incidence of grade 4 adverse events and a 68% (142 of 208) incidence of nausea, vomiting, or diarrhoea, in contrast to higher doses of sertraline (300–400 mg/day), which were associated with a 23% cumulative incidence of grade 4 adverse events and 64% incidence of nausea, vomiting, or diarrhoea. Identifying effective and less toxic antifungal induction regimens could lead to less dependence on completing a full, 14-day course of amphotericin. By contrast, fluconazole monotherapy remains a reality worldwide where amphotericin is unavailable and leads to suboptimal clearance, resistance, and symptomatic relapse. In view of the widespread availability, low cost (US$0.05 per 100 mg tablet wholesale), and extensive safety record of sertraline, the combination of sertraline with fluconazole might offer a more efficacious and cost-effective, all-oral option in such settings. Furthermore, unlike amphotericin and fluconosine, the ability to safely administer sertraline over a prolonged duration would allow antifungal benefits to extend through the consolidation and maintenance phases of therapy.

Unlike previous cohorts, we observed no excess mortality over 12 weeks in people whose CSF culture remained positive at 2 weeks, suggesting the benefit of sertraline might be extended into the consolidation phase due to the additive antimicrobial effects of combination sertraline and fluconazole. Depression is common in this population (77% prevalence), and sertraline would be the obvious antidepressant of choice, if indicated. Additional studies are needed to assess the role of sertraline in consolidation and maintenance phases of therapy, during induction therapy in the absence of amphotericin, and for non-meningitis manifestations of cryptococcosis including asymptomatic cryptococcal antigenaemia identified as part of screening programmes.

A possible, unanticipated benefit of extending adjunctive sertraline beyond the induction period was the observed low incidence of symptomatic recurrence, including both culture-positive cryptococcal relapse and paradoxical immune reconstitution inflammatory syndrome. Although patients were only actively followed up for 12 weeks and passively thereafter, the incidence of about 5% paradoxical immune reconstitution inflammatory syndrome and two late relapse cases appeared to be lower than the 17% immune reconstitution inflammatory syndrome incidence during the COAT trial, and lower than the 25–30% paradoxical cryptococcal-immune reconstitution inflammatory syndrome observed in two cohorts using amphotericin alone as induction therapy with fluconazole 400 mg/day consolidation therapy. The inability to achieve eventual CSF culture sterility is a major risk factor for immune reconstitution inflammatory syndrome and relapse. Improved overall microbiological activity on active and quiescent yeasts might be plausible explanations for the low level of recurrence observed. It is also plausible that sertraline might have immunoregulatory effects on immune activation resulting in a decreased incidence of paradoxical immune reconstitution inflammatory syndrome. The possible decreased immune reconstitution inflammatory syndrome and relapse with adjunctive sertraline is intriguing and requires further investigation in a randomised trial.

The results of our in-vitro susceptibility testing confirm previous studies that provided the impetus for this study, with MICs of 4 μg/mL or less in about 80% of Ugandan isolates, and reported bidirectional synergy with the addition of fluconazole. Synergy might reflect additive mechanisms of antifungal action. Fluconazole targets the enzyme 14α-demethylase, with inhibition of ergosterol synthesis and subsequent membrane disruption. Sertraline, by contrast, inhibits mRNA translation into protein synthesis. For these reasons, the addition of sertraline to standard fluconazole-containing regimens could be ideal for treating persistent or recurrent infections with cryptococcus, factors associated strongly with increased rates of fluconazole resistance.

SSRIs are among the most prescribed drug classes worldwide, and sertraline remains among the most prescribed medications in the USA, with about 44 million prescriptions in 2014. Although inter-person steady state plasma concentrations vary substantially, observed plasma
and rifampin are suspected to lower sertraline levels when co-administered. Our results support these observations, with about 27% lower sertraline plasma concentrations when receiving efavirenz, although the effect of this interaction in the brain is unknown. Only three participants received concurrent rifampin, and sertraline levels were too variable to make firm conclusions. Further analyses of the effect of these medications on sertraline concentrations are needed.

Of particular interest regarding the use of sertraline for fungal meningitis, previous pharmacokinetic studies in animals report that sertraline concentrations are 20–50-times higher in the brain than in blood, and a study of 11 victims of a fatal air crash revealed a mean 21-fold and median 16-5-fold higher concentration in human brain tissue than in blood. Although sertraline is concentrated into the brain tissue, we found low levels in CSF, possibly because sertraline is a lipophilic compound concentrated within the brain parenchyma itself, not CSF. Because amphotericin penetrates poorly into brain parenchyma, the overall benefit of sertraline might be more substantial than the CSF findings suggest. Despite our inability to measure sertraline concentrations in CSF, the inferred pharmacokinetics suggest that brain concentrations of sertraline probably exceed the sertraline MICs reported in vitro when dosed at 400 mg/day. In the presence of at least a two-fold additive effect of fluconazole, therapeutic levels of sertraline in the brain should be achieved in 97% of people when dosed at 400 mg/day, 90% of people dosed at 200 mg/day without ART, and 62% of people dosed at 200 mg/day on efavirenz. Similar therapeutic levels should be achieved in liver, lung, and spleen tissue.

In summary, an improved rate of fungal clearance from the CSF, combined with acceptable concentrations and the additive effects of sertraline used in combination with fluconazole in vitro, suggest that sertraline may be a useful adjunct for the treatment of cryptococcal meningitis with further prevention of paradoxical cryptococcal immune reconstitution inflammatory syndrome and relapse. Given sertraline’s fungicidal properties, safety profile, lack of relevant drug interactions, probable novel mechanism of action, low cost, and excellent brain penetration, sertraline fulfils many of the characteristics required of a new antifungal against cryptococcus. On the basis of the cryptococcus MICs observed and probable brain concentrations of 400 mg/day sertraline, 81% of people would achieve therapeutic sertraline activity in brain tissue. The Adjunctive Sertraline for the Treatment of Cryptococcal Meningitis (ASTRO-CM) randomised clinical trial (NCT01802385) began on March 9, 2015, and is testing if sertraline dosed initially at 400 mg/day has a survival benefit compared with placebo when receiving standard induction therapy of amphotericin B deoxycholate and fluconazole 800 mg/day.

**Contributors**

DRB, DBM, and JR participated in the study concept and design. JR, HWN, KK, LT, AM, AAK, DAW, MA, NCB, SSV, JF, AAI, and KDS participated in the acquisition of data. KHH, BMM, AAI, and DRB participated in the statistical analysis. DRB, DBM, JR, AAI, and KN participated in the interpretation of data. JR, SSV, and BMM participated in initial manuscript drafting. DRB, DBM, JR, KN, and AM participated in critical revisions for intellectual content. DRB, DBM, and JR participated in obtaining funding. DAW participated in administrative, technical, or secretarial work.

**Declaration of interests**

We declare no competing interests.

**ASTRO-CM team members**


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